SYNOPSIS

The thesis entitled “Synthesis of bisindole conjugates and 2-anilinonicotinyl linked oxadiazoles/2-aminobenzothiazoles/triazolobenzothiadiazines as potential anticancer agents” has been divided into four chapters. Chapter-I gives the introduction about cancer chemotherapy, DNA-interactive pyrrolo[2,1-c][1,4] benzodiazepines, benzothiadiazines, bisindoles and 2-anilino nicotinyl conjugates. Chapter-II deals with the design, synthesis and biological evaluation of novel bisindole linked pyrrolo[2,1-c][1,4]benzodiazepines as potential anticancer agents. Chapter-III deals with synthesis of 2-anilinonicotinyl linked 2-aminobenzothiazoles and novel triazolobenzothiadiazines conjugates as apoptosis inducers and their anticancer efficacy. Chapter-IV, Section A comprise of synthesis of 2-anilinonicotinyl linked 1,3,4-oxadiazole derivatives and their biological evaluation as tubulin polymerization inhibitors. Chapter-IV, Section B describes the synthesis of 3,3-diindolyl oxyindoles and their anticancer activity.

Chapter-I: General introduction

Cancer is a disease caused by the malfunctioning of normal cells. It is one of the most feared diseases due to a general perception that it is an indiscriminate and incurable affliction that insidiously attacks people of all cultures and ages. Chemotherapy of the use of chemical agents to destroy the cancer cells is a mainstay in the treatment of malignancies. Though, the classical treatment of cancer, typically involves surgical removal of tumours or destruction by localized radiotherapy, chemotherapy is of utmost importance in order to ensure that all the malignant cells, including any meta-stats are destroyed.

Cancer chemotherapy may also improve both patient survival and well being to variable extent. Thus, there is no doubt an essential role for chemotherapeutic drugs in contemporary clinical oncology. The development of the area of anticancer drug discovery basically reflects an evolution from highly empirical approaches, based on serendipitous findings and testing of randomly selected compounds, to
the current, more focused testing of natural products, rationally synthesized agents, and biological products against panels of well-characterized tumour cell lines or molecular targets. The major categories of chemotherapeutic agents are naturally occurring antitumour antibiotics, DNA interactive agents, enzyme inhibitors such as cyclin-dependent kinase, carbonic anhydrase, tubulin polymerisation, topoisomerase I and II etc. which play a key role in cell division.

The pyrrolo[2,1-c][1,4]benzodiazepines (PBDs) are well known class of antitumour antibiotics with sequence selective DNA binding ability that are derived from various Streptomyces species. The first pyrrolo[2,1-c][1,4]benzodiazepine antitumour antibiotic anthramycin has been described by Leimgruber co-workers in 1963, and since then a number of compounds have been developed on PBD ring system leading to DNA binding ligands. Their mode of interaction with DNA has been extensively studied and it is considered unique as they bind within the minor groove of DNA. These compounds exert their biological activity by covalently binding to the C2-amino group of guanine residue in the minor groove of DNA through the imine or imine equivalent functionality at N10-C11 of the PBD moiety.

E7010 is a novel sulfonamide, which inhibits tubulin polymerisation. This compound causes cell cycle arrest and apoptosis in M phase that exhibits good in vivo antitumour activity against several rodent and human tumours and is in phase II clinical studies. N-Phenyl nicotinamides are another new class of apoptosis inducers that are known to arrest cells in G2/M phase and SAR studies indicated that the 3-pyridyl group is very important for their activity. Recently in this laboratory a series of 2-anilinonicotinyl linked sulfonyl hydrazide derivatives (Fig.
1) have been synthesized which exhibited anticancer activity against a panel of cancer lines.

![Figure 1. Compounds exhibiting anticancer activity.](image)

Sulfonamides have been clinically used for many years and found to posses a large number of biological activities, including antibacterial and anticancer. Benzothiadiazine ring system has been considered as cyclic sulfonamides and these derivatives have shown strong activity against several cancer cell line. In the last decade triazole derivatives have attracted much interest for the development of potent anticancer agents. Lin and co-workers reported 1,2,4-triazole-3,5-diamine analogues as novel and potent anticancer cyclin-dependent kinase (CDK) inhibitors. Further, triazole derivatives possess anticancer activity by inhibition of tubulin polymerization. Further another novel series 10-substituted 5,5-dioxo-5,10-dihydro[1,2,4]triazolo[4,3-b][1,2,4] benzothiadiazine coupled with sulfanyl acetamido benzothiazole derivatives (Fig. 2) exhibited significant growth inhibition against RPMI-8226 (leukemia) and HOP-62 (lungs) cell lines.

![Figure 2. Triazolobenzodithiazine derivatives as anticancer agents.](image)
Bisindole alkaloids comprise of two indole moieties connected to each other via heterocyclic units exhibit a wide spectrum of pharmacologic activities such as antibacterial, antifungal, cytotoxic, antitumour, antiviral, antimicrobial, and anti-inflammatory activities as well as binding to α1-adrenergic receptor. Vinblastine and vincristine are vinca alkaloids isolated from *Madagascar periwinkle* plant and currently used as chemotherapeutic agents, the mode of action of these drugs is it binds tubulin, thereby inhibiting the assembly of microtubules and its closely related semisynthetic antitumor bisindole analogs namely KARs (Fig. 3) has also shown to be inhibitors of tubulin polymerization and exhibit anticancer activity with lower toxicity than the naturally occurring ones in neuroblastoma cells.

![Chemical structures of vinblastin, vincristine and semisynthetic bisindole derivatives KARs.](image)

**Figure 3.** Chemical structures of vinblastin, vincristine and semisynthetic bisindole derivatives KARs.

**Chapter-II:**

**Synthesis, anticancer activity and apoptosis inducing ability of bisindole linked pyrrolo[2,1-c][1,4]benzodiazepine conjugates**

It has been considerable interest in the past few years to design and synthesize cross-linking agents, particularly based on pyrrolobenzodiazepines (PBDs). Pyrrolo[2,1-c][1,4]benzodiazepines are of current interest due to their ability to recognize and subsequently form covalent bonds to specific base sequences of double stranded DNA. PBD antitumour antibiotics bind covalently to the N2 of guanine at purine-guanine-purine sites in the minor groove of DNA.
3,3′-Diindolylmethane (DIM) is an anticancer agent naturally formed during the autolytic breakdown of glucobrassicin, which is present in food plants of the *Brassica genus*. DIM, can reduce the incidence of different classes of reproductive tissue tumors. Further DIM induces programmed cell death of human breast cancer cells as well as endometrial tumour cells.

The objective of the present work is the synthesis of new bisindole linked pyrrolo[2,1-c][1,4]benzodiazepines, and study their DNA binding affinity as well as *in vitro* anticancer activity and the most potent compounds were taken up for detailed studies on MCF-7 cell line.

Synthesis of C8-linked bisindole-PBD conjugates has been carried out by employing the commercially available vanillin (1) as the starting material. Oxidation of vanillin to form the corresponding carboxylic acid followed by acid-catalyzed esterification with methanol provided methyl benzoate in quantitative yield. This is followed by benzylation and nitration by employing the literature method to provide the 4-benzyloxy-5-methoxy-2-nitrobenzoic acid (6). Later this has been coupled to L-proline methyl ester to afford compound 7, which upon reduction with DIBAL-H produces the corresponding aldehyde 8. This product upon protection with EtSH/TMSCl gives compound 9, which on debenzylation provides the hydroxyl intermediate compound 10 which upon etherification by dibromoalkanes provide 11a-c (Scheme 1).
Reagents and conditions: (i) NH$_2$SO$_3$H, NaClO$_2$, H$_2$O, rt, 2 h, 90%; (ii) H$_2$SO$_4$, MeOH, reflux, 4 h, 85%; (iii) benzylbromide, K$_2$CO$_3$, acetone, reflux, 24 h, 92%; (iv) SnCl$_4$, fuming HNO$_3$, CH$_2$Cl$_2$, 5 min, -25 °C, 78%; (v) 2N LiOH, MeOH, H$_2$O, THF (1:1:3), rt, 12 h, 83%; (vi) SOCl$_2$, C$_6$H$_6$, L-proline methylester hydrochloride, THF-H$_2$O, 1-2 h, rt, 85%; (vii) DIBAL-H, CH$_2$Cl$_2$, 1-1.3 h, -78 °C, 65%; (viii) EtSH, TMSCl, CH$_2$Cl$_2$, 8-12 h, rt, 72%; (ix) BF$_3$OEt$_2$, EtSH, CHCl$_3$, rt, 8 h, 75%; (x) dibromoalkane, K$_2$CO$_3$, acetone, reflux, 24 h, 92-96%.

The preparation of bisindole intermediates (14a,b) has been accomplished by the reaction of indole (12) with substituted hydroxy benzaldehydes (13a,b) in acetonitrile and aluminium triflate as a catalyst to afford the phenol substituted derivatives (14a,b) as illustrated in Scheme 2.
Reagents and conditions: (i) Al(OTf)$_3$, dry acetonitrile, rt, 1-2 h.

Synthesis of C8-linked bisindole-PBD conjugates (17a-f) has been carried out from the (2S)-N-[4-[3-bromoalkoxy-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal (11a-c), These upon etherification with the phenolic precursors (14a,b) using K$_2$CO$_3$ in acetone provided the corresponding nitrothioacetals 15a-f). These nitrothioacetals 15a-f are reduced to the amino thioacetals 16a-f by employing SnCl$_2$.2H$_2$O in refluxing MeOH and then cyclized by treatment with HgCl$_2$ and CaCO$_3$ in MeCN-H$_2$O to yield the target products 17a-f (Scheme 3).
Reagents and conditions: (i) \text{K}_2\text{CO}_3, \text{acetone, reflux, 24 h, 85-90\%} ; (ii) \text{SnCl}_2.\text{2H}_2\text{O, MeOH, 4 h, reflux, 75\%} ; (iii) \text{HgCl}_2, \text{CaCO}_3, \text{CH}_3\text{CN/H}_2\text{O, 12 h, rt, 55-60\%}.

The thermal denaturation studies showed that hybrid agents (17a-f) possess good DNA binding ability compared to DC-81. These conjugates 17a-f were evaluated for their anticancer activity in selected human cancer cell lines. All these compounds 17a-f exhibited anticancer potency with GI$_{50}$ values ranging from 0.11 to 30.8 µM, whereas the positive controls adriamycin and DC-81 demonstrated GI$_{50}$ values in the range of <0.1-14.7 µM and 0.10 to 2.37 µM concentrations, respectively. Interestingly all the compounds are active in human breast cancer cell line (MCF-7) with GI$_{50}$ values ranging from 0.14 to 2.01 µM. The most potent compounds 17b and
were taken up for detailed studies on MCF-7 cell line. Cell cycle effects were examined apart from investigating the inhibition of tubulin polymerization for compound 17e at 2 μM. FACS analysis showed that at higher concentrations (4 and 8 μM) there was an increase of sub-G1 phase cells and decrease of G2/M phase cells, thus indicating that compounds 17b and 17e are effective in causing apoptosis in MCF-7 cells. It was also observed that compounds 17b and 17e showed the down regulation of histone deacetylase protein levels such as HDAC-1, 2, 3, 8 and increase in the levels of p21, followed by apoptotic cell death. *(Bioorg. Med. Chem. Lett. 2011, Communicated).*

Chapter-III

**Synthesis of 2-anilino nicotinyl linked 2-aminobenzothiazoles and [1,2,4] triazolo [1,5-b][1,2,4]benzothiadiazine conjugates as potential mitochondrial apoptosis inducers**

Benzothiazoles are small synthetic molecules that contain a benzene ring fused to a thiazole ring and known for different biological properties like antimicrobial, anticancer and as amyloidal, antirheumatic, antiglutamate agents. The other class of benzothiazoles which are closely related to 2-(4-aminophenyl) benzothiazoles have also shown good antitumour activity. The replacement of phenyl group with other functional groups like 6-ethoxy-1,3-benzothiazole-2-sulfonamide (ethoxazolamide) has been also found to possess potent antitumour activity by inhibiting carbonic anhydrase.

The objective of the present work is the synthesis, biological evaluation of 2-anilino nicotinyl linked 2-amino benzothiazoles and novel [1,2,4]triazolo[1,5-b] [1,2,4]benzothiadiazine conjugates as anticancer agents and apoptosis inducers.

2-Chloronicotinic acid ethylester (1) and substituted anilines (2a-g) were refluxed in ethylene glycol to give the coupled product of 2-anilinonicotinic acid esters (3a-g), which on treatment with 2N NaOH solution in ethanol afforded 2-anilinonicotinic acids (4a-g) in quantitative yields. The synthesis of the final
products (6a-n) was carried out by the reaction of corresponding 2-anilinonicotinic acid with 6-substituted 2-amino benzothiazoles (5a,b) with EDCI/HOBt in dry DMF as solvent (Scheme 1).

Reagents and conditions: (i) ethylene glycol, 160 °C, 6h; (ii) 2N NaOH, ethanol, reflux 2h; (iii) EDCI/HOBt, dry DMF 0 °C-rt, 8h.

The preparation of key intermediates 3-hydrazone-4-alkyl/aryl-4H-1,2,4-benzothiadiazine 1,1-dioxide (10a-b) and 3-chloro-4-alkyl/aryl-4H-1,2,4-
benzothiadiazine 1,1-dioxide (9a-b) has been carried out by synthetic sequence illustrated in Scheme 2. The treatment of chlorosulfonyl isocyanate with N-alkyl/aryl aniline (7a–b) in nitromethane, followed by cyclization with aluminium chloride, provides 4-alkyl/aryl-2H-1,2,4-benzothiadiazine-3(4H)-one 1,1-dioxide (8a-b). This upon chlorination with PCl5 affords 3-chloro-4-alkyl/aryl-4H-1,2,4-benzothiadiazine 1,1-dioxide (9a–b), which on treatment with hydrazine hydrate in chloroform yields 3-hydrazino-4-alkyl/aryl-4H-1,2,4-benzothiadiazine 1,1-dioxide (10a–b).

**Scheme 2**

Reagents and conditions: (i) CH3NO2, 0 °C, rt, 30 min; (ii) AlCl3, 120 °C, 30 min; (iii) PCl5, 190 °C, 30 min; (iv) N2H4.H2O, CHCl3, 0 °C, rt, 1-2 h.

The synthesis of triazolo fused benzothiadiazines (11a-j) was carried out by refluxing 3-hydrazino-4-methyl/phenyl-4H-1,2,4-benzothiadiazine1,1-dioxides (10a,b) and 2-anilino nicotinic acids (4a-i) in phosphorous oxychloride (Scheme 3).
The synthesized compounds were evaluated for their antiproliferative activity in different cancer cell lines further we explored the cytotoxic potential of active compounds (6h, 6i, 6j, 6k, 6n and 11e) were tested for their cell viability by MTT method at the indicated concentrations for 48h. Most of these compounds were found to be cytotoxic in human leukemia HL-60 cells and their IC$_{50}$ values range from 0.08-0.7 µM. Compound 6i showed highest cytotoxicity with IC$_{50}$ value of 0.08 µM. These compounds showed limited cytotoxicity to cervical cancer (SiHa, CV-1) cells. Further investigation on the possible mechanism of these compounds underlying the induction of apoptosis, antiproliferation, cell cycle perturbations, enhancement of reactive oxygen species and activation of caspases -3,-8,-9, also signals leading to activation of a variety of gene products such as NF-kb inhibition, Survivin inhibition, Hsp-90 inhibition, PARP cleavage proteins have also been investigated which are important in the regulation and execution of apoptosis.
induced by various stimuli. Moreover scanning electron microscopy studies also confirmed that compound 6i treated cells after 12h observed decrease in size, smoothening of surface and blebbing of plasma membrane in majority of cells which is generally observed in apoptotic bodies, it is also confirmed by fluorescent microscopic analysis also. (Chem. Med. Chem. Communicated).

Chapter-IV Section A:
2-Anilinonicotinyl linked 1,3,4-oxadiazole derivatives: synthesis, antitumour activity and inhibition of tubulin polymerisation

Oxadiazoles are an important class of heterocyclic compounds with a broad range of biological activities. These five-member heterocycles are also useful intermediates in organic synthesis and widely employed as electron transporting and hole-blocking materials Moreover, it is considered that the presence of toxophoric –N-C–O–linkage is responsible for their potent pharmacological activity. Further, 1,3,4-oxadiazole heterocyclics are very good bioisosters of amide and ester functionalities with substantial improvement in biological activity by participating in hydrogen bonding interactions with different receptors. The biological activity of these compounds depending upon the substituents and positions of heteroatoms namely 1,2,4- and 1,3,4-oxadiazoles are known to possess various biological properties such as antiviral, inhibition of tyroinosine, anti-inflammatory, antibacterial, antitubercular, including anticancer activity.

The present work describes the synthesis and biological evaluation of 2-anilinonicotinyl linked oxadiazoles as anticancer agents and tubulin inhibitors.

The synthesis of oxadiazoles has been carried out by reaction of 2-chloronicotinic acid ethylester (1) and substituted anilines (2a-d) were refluxed in ethylene glycol to provide the coupled product of 2-anilinonicotinic acid esters (3a-d), which on treatment with hydrazine hydrate in ethanol afforded 2-anilinonicotinic acid hydrazides (4a-d) in quantitative yields (Scheme 1). Treatment of 4a-d with substituted aryl isothiocyanates afforded the corresponding thiosemicarbazides (5a-
m) by employing the literature procedure. The synthesis of the final products (6a-m) was carried out by the reaction of thiosemicarbazides (5a-m) in pyridine and tosyl chloride using THF as the solvent under reflux conditions.

Reagents and conditions: (i) substituted anilines (2a-d), ethylene glycol, 160 °C, 8h; (ii) NH2NH2.H2O, ethanol, reflux 6h; (iii) aryl isothiocyanates, dry benzene, rt, 8 h; (iv) Tscl, pyridine, THF, reflux 2-3 h.

A new series of 2-anilino substituted nicotinyl 1,3,4-oxadiazoles was synthesized and evaluated for their antiproliferative and inhibition of tubulin polymerization activities. These compounds exhibited significant anti proliferative
activity with GI$_{50}$ values ranging from 4.57-97.0 µM. Most of the compounds were specifically active in cervix cancer cell line with GI$_{50}$ values 5.1-8.6 µM. The lead compound 6m exhibits significant antiproliferative activity with GI$_{50}$ values ranging from 4.57-10.69 µM and showed to be potent to inhibit tubulin polymerization in both in vitro assay as well as in cells as observed by the increase in the ratio of soluble to polymerized tubulin. Moreover, this compound also possesses reasonably good aqueous solubility with ClogP value of 3.19. Further studies indicated that cell cycle arrest at G2/M phase followed by the activation of caspases as the mechanism of cytotoxicity for compound 6m. The compound 6m has shown comparable inhibition of tubulin polymerization to related compounds. These mechanistic studies have provided an insight for the further development of such tubulin inhibitors as potential anticancer agents. (Med. Chem. Comm. 2011, accepted)

Chapter-IV Section B:

Synthesis of 3,3-diindolyl oxyindoles efficiently catalyzed by FeCl$_3$ and their in vitro evaluation for anticaner activity

There has been tremendous interest in developing highly efficient transformations for the synthesis of organic products, as well as biologically active molecules with potential pharmaceutical importance from commercially available compounds. There is also need for synthetic chemists to find new, efficient, and strategically important processes, which are environmentally benign and in short period of times with high yields and simple work up procedures. In many cases, the inspiration for developing the new methodology arises from a consideration of the structural features of such targets.

Oxindoles are known to posses antibacterial, antiprotosoal, and anti-inflammatory activities and are also patented as PR (progesterone receptors) agonists. The naturally occurring oxindole derivative convolutamydine has been found to exhibit potent activity in the differentiation of HL-60 human promylocytic leukemic cells. Various indolin-2-one derivatives such as sutent a drug approved by
FDA in 2006 used to treat advanced kidney cancer (advanced renal cell carcinoma). Several 2-indolone imino derivatives have been evaluated as antitumour and antiangiogenic agents. A hybrid pharmacophore approach was designed and synthesized isatin–benzothiazole analogs which exhibiting anticancer activity against breast cancer. A series of substituted 3,3-diphenyl-1,3-dihydroindol-2-ones were synthesized from the corresponding isatins. One of the compound m-tert butyl and o-hydroxy substituted diphenyl oxindole exhibiting Ca²⁺-depletion-mediated inhibition of translation initiation.

![Figure 4. Indolin-2-ones derivatives with anticancer activity.](image)

On the basis of previous studies on oxyindoles and bisindolyl methanes, it was speculated that combining the structural characteristics of both these moieties could produce considerable enhancement in the anticancer activity of such compounds. This prompted us to design molecules wherein the two indolyl moieties are linked to the same carbon atom of an indoline-2-one. Keeping this objective in mind bisindole derivatives, namely 3,3-diindolyl oxyindoles have been prepared and evaluated for anticancer activity.

We first attempted the reaction of isatin with indole and reaction was carried out using 5 mol% anhydrous FeCl₃ in acetonitrile for less than one hour at room temperature. Later considering the encouraging results we expanded our attention towards a various indoles and isatins to produce 3,3-diindolyl oxyindole derivatives in high yields. Both electron donating and electron withdrawing substituents on
indoles, reacted effectively with isatin under the same reaction conditions (Scheme 1).

All the synthesized compounds were evaluated for *in vitro* cytotoxicity against a panel of five human cancer cell lines including lung (A-549), CNS (SK-N-SH), breast (MCF-7), liver (HepG-2) and prostate (DU145) by using MTT assay. IC$_{50}$ values (in μM), which is the concentration required to inhibit 50% of cell viability by the test compounds after exposure to cells, have been determined. Results indicate that most of the compounds displayed good anticancer potency against the cell lines tested in the assay. Compounds 3c, 3d, 3f and 3k showed excellent anticancer potency with IC$_{50}$ values of 2.2, 1.2, 3.6 and 3.6 μM respectively, against prostate cancer cell line DU145 cell line in comparison to structurally related analogues, (GI$_{50}$ = 1.08-12.51 μM) against DU145 cell line. Notably, some of the compounds displayed marked potency selectively against prostate cancer cell line and CNS cancer cell line with IC$_{50}$ value up to 1.2 and 2.8 μM, respectively. It is expected that these compounds which resemble with indole derivatives could exhibit anticancer activity by acting as HDAC or CDK inhibitors. *(Bioorg. Med. Chem. Lett. 2010, 16, 7804–7810).*